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Helminth antigen-based strategy to ameliorate inflammation in an experimental model of colitis

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Summary

Inflammatory bowel disease (IBD) is the most common and serious chronic inflammatory condition of the gut. Among the distinct T helper (Th) cell subsets, a Th1 type response is associated predominantly with Crohn's disease (CD) while helminth infections generate a strong Th2 type response. IBD is most prevalent in developed countries but rare in countries where infections with helminths are common. Thus, it has been hypothesized that infection with helminth infection influence the development of CD and recent clinical and experimental studies suggest strongly a beneficial role of helminth infection in IBD. In the present study we examined the effects of rectal submucosal administration of helminth antigens on subsequent experimental colitis. Mice were treated with *Trichinella spiralis* antigens prior to the induction of dinitrobenzenesulphonic acid (DNBS)-induced colitis and were killed 3 days post-DNBS to assess colonic damage macroscopically, histologically and by myeloperoxidase (MPO) activity, inducible nitric oxide synthase (iNOS) and cytokine levels. Previous treatment with *T. spiralis* antigens reduced the severity of colitis significantly, as assessed macroscopically and histologically, and reduced the mortality rate. This benefit was correlated with a down-regulation of MPO activity, interleukin (IL)-1β production and iNOS expression and an up-regulation of IL-13 and transforming growth factor-β production in colon. These results clearly show a beneficial role of local treatment with helminth antigens for experimental colitis and prompt consideration of helminth antigen-based therapy for IBD instead of infection with live parasites.

Keywords: helminth, IBD, inflammation, immunomodulation, *Trichinella spiralis*

Introduction

The human inflammatory bowel diseases (IBDs) are chronic, recurrent intestinal disorders of complex pathogenesis, which are represented mainly by Crohn's disease (CD) and ulcerative colitis (UC). Both CD and UC have a prevalence range of 10–200 per 100 000 individuals per year in North America and Europe [1]. IBD can begin relatively early in life and persist for long periods, leading to substantial morbidity and decreased quality of life [2]. The causes of IBD are unknown, but epidemiological and laboratory work suggests that environmental and genetic factors, which are associated with dysregulation of the mucosal immune system, are important in the pathogenesis of IBD [3–6].

The IBD is not distributed evenly worldwide. IBD is common in developed countries and is rare in less-developed countries where helminth infections are common [7]. The rarity of IBD in less-developed countries cannot be explained on the basis of genetics alone, as descendants of immigrants from such countries acquire the higher risk of IBD of the adopted developed country [8,9]. IBD is more common in urban *versus* rural areas [10] and less common in people who have jobs exposing them to 'dirt' and exposure to a variety of environmental antigens and pathogens [11]. These observations suggest strongly an important role of environmental factors in the expression of IBD.

T cells are critical in many immune responses, including those associated with IBD and helminth infection. Among the distinct T helper (Th) cell subsets, a Th1 type response is associated predominantly with CD, while helminth infections prototypically generate a strong Th2 response. The reciprocal cross-regulation between Th1 and Th2 cells

suggests that mobilization of a Th2 response by helminths could prevent or reduce the effects of Th1-mediated diseases. Considering the contrasting geographical distribution and immune responses of helminth infections and CD, it is hypothesized that an environmental factor such as helminth infection may influence the development of CD. This has led to the development of the 'hygiene hypothesis' of IBD; that is, that IBD occurs more commonly in societies where the prevalence of chronic enteric infestation is low [7]. Previously we have shown that prior infection with the helminth parasite, *Trichinella spiralis*, ameliorates subsequent hapten-induced colitis in mice and this was associated with a down-regulation of the Th1 response [12]. Recent clinical studies provide evidence that *Trichuris suis* ova therapy is effective in treating both CD and UC without any adverse effects [13,14]. The outcome of these trials supports further the concept that de-worming the population has led to the rising prevalence of IBD. Taken together, there is abundant evidence of immunomodulatory functions following infection with helminths and to support the therapeutic potential of helminth infection in the treatment of immunologically driven intestinal inflammation. However, the ingestion of live parasites or eggs as a therapeutic modality may not be attractive to patients, and may not be acceptable to many. To counter this hindrance, in this study we investigated an alternative strategy by examining whether the submucosal delivery of helminth antigen achieves the same therapeutic benefit utilizing the well-defined model of hapten (dinitrobenzenesulphonic acid; DNBS)-induced colitis in mouse. Our results demonstrate that rectal submucosal treatment with *T. spiralis* antigens prior to the induction of DNBS-induced colitis reduced significantly the severity of colitis both macroscopically and histologically, together with a reduction in mortality rate. This was correlated with a down-regulation of myeloperoxidase (MPO) activity, interleukin (IL)-1β production and inducible nitric oxide synthase (iNOS) and an up-regulation of IL-13 and transforming growth factor (TGF)-β production in the colon.

Materials and methods

Animals

C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME, USA) were kept in sterilized, filter-topped cages under specific pathogen-free conditions and fed autoclaved food; only male mice aged 8–10 weeks were used. The protocols employed were in accordance with guidelines drafted by the McMaster University Animal Care Committee and compiled with the Canadian Council on the Use of Laboratory Animals.

Preparation of *T. spiralis* antigen and rectal submucosal administration of antigen

Antigen was prepared from *T. spiralis* frozen skeletal muscle larvae using the method of Russell and Castro [15]. Briefly, muscle larvae were obtained after artificial digestion of infected mouse carcasses using a solution containing pepsin and hydrochloric acid. The larvae were washed 10 times by sedimentation in phosphate-buffered saline (PBS), and after washing the larvae were maintained on ice in PBS, homogenized and centrifuged. After centrifugation the antigen-containing supernatant was collected and the concentration of protein in the antigen-containing supernatant was determined by a commercially available protein assay kit (Bio-Rad, Hercules, CA, USA).

Mice were treated with *T. spiralis* antigen (50 μg or 100 μg in 30 μl PBS) or with PBS submucosally in rectum 5 days before the induction of colitis by DNBS. To confirm that the solution was delivered properly in submucosa, trypan blue dye was added into the solution (1–2%) so that we could see the solution through the mucosa. Successful administration was accepted when there was elevation of mucosa and blue solution was observed through the mucosa. The elevation of mucosa indicated that the injected antigen solution reached into the submucosal layer and raised the mucosal layer.

Induction of colitis

Colitis was induced by intracolonic administration of DNBS (ICN, Aurora, OH, USA), as described previously [16]. In brief, a stock solution of DNBS was made by dissolving 50 mg of DNBS per ml of 50% ethanol. Mice anaesthetized with 2% enflurane were injected in the distal 4 cm of the colon with 100 μl of this solution, containing 5 mg of DNBS, using a 1-ml tuberculin syringe (Becton Dickinson, Franklin Lakes, NJ, USA) and flexible PE90 tubing (Clay Adams, Parsippany, NJ, USA). The mice were given 8% sucrose in 0·2% saline in their drinking water to prevent dehydration after DNBS administration. Mice were killed on day 3 post-DNBS.

Assessment of colonic damage

The colon was removed and opened longitudinally, and the damage was assessed macroscopically and histologically using previously published criteria [16]. Briefly, the macroscopic criteria included macroscopic mucosal damage (assessed with a scale), thickening of the colonic wall, the presence of adhesions between the colon and other intra-abdominal organs, the consistency of faecal material (as an indicator of diarrhoea) and the presence of hyperaemia. Microscopic criteria for damage and inflammation were investigated by light microscopy on haematoxylin and eosin-stained histological sections obtained from gut segments taken from a region of the inflamed colon immediately adjacent to gross macroscopical damage. Histological criteria were based on the following: degree of mucosal architectural changes, cellular infiltration, goblet cell depletion and presence of crypt abscess. Macroscopic and histological damage were recorded and scored for each mouse by two different investigators who were blinded to the treatment condition.

The MPO activity

The degree of colonic inflammation was investigated by assay of MPO activity. Three days after the intrarectal administration of DNBS, the colon was removed, snap-frozen in liquid nitrogen and stored at −70°C. Samples were weighed and MPO was measured using a technique described previously [17]. MPO activity is reported as units of MPO per mg of wet tissue. One unit of MPO was defined as the quantity of enzyme required to convert 1 μmol of hydrogen peroxide to water in 1 min at room temperature.

Evaluation of intestinal tissue cytokine levels

Frozen intestinal tissues were homogenized in lysis buffer containing protease inhibitor cocktail (Sigma, St Louis, MO, USA). The homogenates were freeze-thawed three times and centrifuged, and the supernatant was collected and stored at −20°C until analysed.

The IL-1β, IL-4, IL-10, IL-13, interferon (IFN)-γ and TGF-β levels in the supernatant were measured by an enzyme immunoassay technique using a commercially available kit purchased from R&D Systems (Minneapolis, MN, USA). The concentration of protein in the intestinal tissue was determined by a commercially available DC protein assay kit (Bio-Rad), and the amount of cytokines in the tissues were expressed per mg of tissue protein.

Detection of iNOS by reverse transcription–polymerase chain reaction

Total RNA fractions were prepared from freshly isolated colon tissue based on a previously described guanidium isothiocyanate method [18]. The concentration of RNA was determined by measuring absorbance at 260 nm, and its purity was confirmed using the ratio of absorbency at 260 nm to that at 280 nm. RNA was stored at −70°C until it was used for reverse transcription–polymerase chain reaction (RT–PCR). mRNA was then reverse-transcribed as described previously to yield cDNA, and the cDNA was amplified by PCR using gene-specific primers.

Fifty-nanogram aliquots of cDNA were then mixed with 20 pmol each of upstream (5′-TTCCGAAGTTTCTGGCA GCA-3) and downstream (5′-ATAGGAAAAGACTGCAC CGAAGAT-3′) primers for iNOS [19]. The housekeeping gene β-glucoronidase was used as the positive control, and to detect it the upstream (5′-TGTATGTGGTCTGTGG CCAA-3′) and downstream primers (5′-TCTGCTCCATA CTCGCTCTG-3′) were used [20]. PCR was performed in 50-μl volumes containing deoxynucleoside triphosphate (200 μM), $MgCl_2$ (1·5 mM) and 2·5 U of *Taq* polymerase (Gibco BRL, Carlsbad, CA, USA) with the corresponding buffer and distilled water. Messages for iNOS and β-glucoronidase were co-amplified using the following parameters: denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 60 s. The PCR products were loaded onto a 2·5% agarose gel and then visualized under ultraviolet light after ethidium bromide staining.

Investigation of iNOS expression by immunohistochemistry

Immunohistochemical studies of iNOS expression were performed on formalin-fixed, paraffin-embedded samples. Sections were deparaffinized in CitriSolv (Fisher Scientific, Ontario, Canada) and rehydrated through a graded series of ethanols and PBS. Endogenous peroxide was blocked by incubation in peroxidase-blocking reagent (DakoCytomation, Ontario, Canada) for 15 min. After washing, sections were subjected to antigen retrieval in citrate buffer following microwave heating. Following blocking of non specific binding with 1% bovine serum albumin in PBS, the sections were incubated with polyclonal rabbit antibody against targeted against the carboxyl terminus of mouse iNOS (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1 h at room temperature). After washing, sections were incubated with Envision (horseradish peroxidase-coupled anti-rabbit secondary reagent; DakoCytomation) for 30 min. The sections were developed with 3,3′-diaminobenzidine and counterstained with Meyer's haematoxylin. The areas stained with anti-iNOS antibody were measured by ImageJ software.

Statistical analysis

Data were analysed using Student's *t*-test, with a *P*-value of $< 0{\cdot}05$ considered significant. All results are expressed as the mean ± standard error of the mean.

Results

Macroscopic evaluation of the colon of mice 3 days after the administration of 5 mg of DNBS in PBS-treated mice revealed massive ulceration, thickening of the colonic wall, hyperaemia and severe adhesions between the colon and other organs. This was accompanied by a 10% mortality rate. In contrast, rectal submucosal treatment with 100 μg of *T. spiralis* antigen 5 days prior to DNBS-induced colitis reduced significantly the severity of DNBS-induced colitis. These *T. spiralis* antigen-treated mice showed significantly less mucosal damage, less thickening of the colonic wall and fewer adhesions (Fig. 1). There was no mortality in mice treated with *T. spiralis* antigen before the administration of DNBS. Histological examination of the colon in PBS-treated mice after DNBS treatment revealed an intense granulocyte infiltrate extending throughout the mucosa and submucosa, often involving the muscularis propria. There was also marked mucosal damage associated with colonic goblet cell depletion. Rectal submucosal administration of 100 μg of *T. spiralis* antigen in mice prior to induction of colitis by DNBS

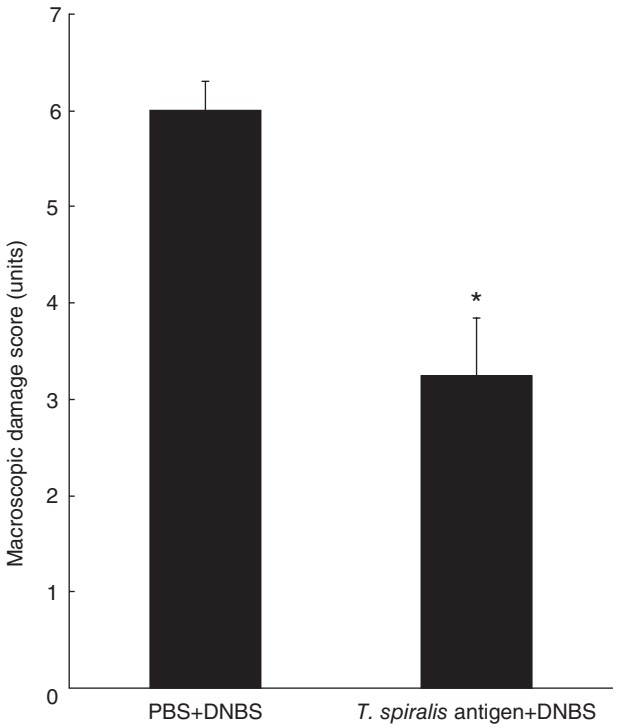


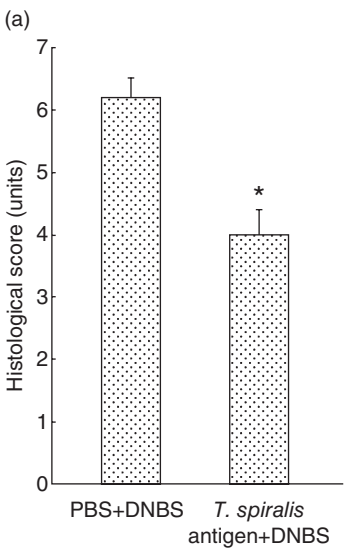
Fig. 1. Macroscopic damage score following dinitrobenzenesulphonic acid (DNBS) administration in *Trichinella spiralis* antigen-treated and phosphate-buffered saline (PBS)-treated mice. C57BL/6 mice were treated with *T. spiralis* antigen (100 μg in 30 μl PBS) or with equal volume of PBS submucosally in the rectum 5 days before the induction of colitis by DNBS. Colitis was induced by intracolonic administration of DNBS (5 mg) and macroscopic damage was evaluated on day 3 post-induction of colitis. Each bar represents the mean ± standard error of the mean. *Significantly lower than PBS-treated mice; $n = 6–8$ mice per group.

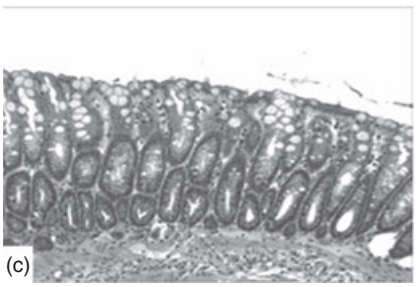
caused a significant improvement of the histological score on day 3 post-DNBS administration (Fig. 2), and this was accompanied by less mucosal damage, less goblet cell depletion and less cellular infiltration into the mucosa and submucosa. Treatment with 50 μg of *T. spiralis* antigen prior to the induction of DNBS-induced colitis had no significant effect on the macroscopic or histological scores of DNBS-induced colitis.

Consistent with the reduced macroscopic and histological scores, we also observed a down-regulation of MPO activity in *T. spiralis* antigen (100 μg)-treated mice. After 3 days of DNBS administration, there was significantly lower MPO activity after DNBS administration in mice treated with *T. spiralis* antigen compared with the mice treated with PBS before the induction of colitis (Fig. 3a). Treatment with the lower dose of antigen (50 μg) had no significant effects on MPO activity following DNBS administration.

Next, we investigated colonic tissue levels of IL-1β, IL-4, IL-13, IFN-γ and TGF-β in both *T. spiralis* antigen- (100 μg) and PBS-treated mice after DNBS administration. As shown in Fig. 3b, similar to MPO activity there was a significantly lower amount of colonic tissue IL-1β on day 3 post-DNBS in mice treated with *T. spiralis* antigen (100 μg) compared with mice treated with PBS before DNBS administration. Treatment with the lower dose of antigen (50 μg) had no significant effects on IL-1β production following DNBS administration. We failed to detect colonic tissue IL-4, IL-10 and IFN-γ on day 3 after DNBS in both antigen- and PBS-treated mice. However, we observed a significantly higher amount of IL-13 in colonic tissue of *T. spiralis* antigen-treated mice after DNBS administration compared with that in PBS-treated mice (Fig. 4a). In addition, we observed a significantly higher amount of TGF-β in the colonic tissues

Fig. 2. Histological assessment of the colon in dinitrobenzenesulphonic acid (DNBS) colitis in *Trichinella spiralis* antigen-treated and phosphate-buffered saline (PBS)-treated mice. Mice were treated with *T. spiralis* antigen (100 μg in 30 μl PBS) or with PBS submucosally in the rectum 5 days before the induction of colitis by DNBS. Colitis was induced by intracolonic administration of DNBS (5 mg) and histological investigations were performed on day 3 post-induction of colitis. (a) Histological damage score. (b) Light micrograph of haematoxylin and eosin-stained colonic section from PBS-treated mouse on day 3 post-DNBS. (c) Light micrograph of haematoxylin and eosin-stained colonic section of *T. spiralis* antigen-treated mouse on day 3 post-DNBS. Each bar represents the mean ± standard error of the mean. *Significantly lower compared with PBS-treated mice. $n = 6–8$ mice per group.





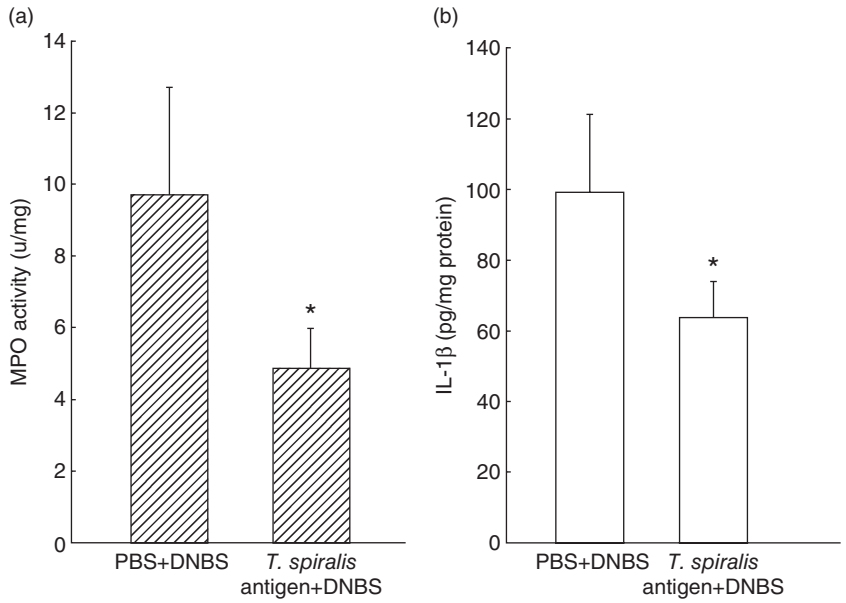


Fig. 3. Measurement of myeloperoxidase (MPO) activity and interleukin (IL)-1β levels in the colonic tissues of *Trichinella spiralis* antigen-treated and phosphate-buffered saline (PBS)-treated mice following dinitrobenzenesulphonic acid (DNBS) administration. Mice were treated with *T. spiralis* antigen (100 μg in 30 μl PBS) or with PBS submucosally in the rectum 5 days before the induction of colitis by DNBS. Colitis was induced by intracolonic administration of DNBS (5 mg) and (a) MPO activity and IL-1β levels (b) in the colon were evaluated on day 3 post-administration of DNBS. Each bar represents the mean ± standard error of the mean of five animals. *Significantly lower compared with PBS-treated mice.

of mice treated with *T. spiralis* antigen before the induction of colitis by DNBS compared with the mice treated with PBS before the induction of colitis (Fig. 4b). We also observed up-regulation of IL-13 and TGF-β levels in colonic tissues of mice which received *T. spiralis* antigen (100 μg) only 8 days after administration of antigens (IL-13 levels in colonic tissues of antigen-treated *versus* PBS-treated mice were 9·8 ± 3·1 pg/mg of protein *versus* 0 ± 0 respectively; TGF-β levels in colonic tissues of antigen-treated *versus* PBS-treated mice were 17·4 ± 8·6 pg/mg of protein *versus* 0 ± 0 respectively).

As NO is an important component of immune and inflammatory responses we next investigated whether the treatment with *T. spiralis* antigen has any effect on NO production in subsequent DNBS-induced colitis. For investigating the effect on NO production with submucosal rectal antigen treatment, colonic tissue samples were studied for the expression of iNOS by investigating iNOS mRNA by RT–PCR and by immunohistochemistry using anti-iNOS polyclonal antibody. We observed a down-regulation of iNOS expression by both RT–PCR (Fig. 5) and immunohistochemistry (Fig. 6) in the colonic tissues of mice treated with *T. spiralis* antigens compared with the mice treated with PBS prior to induction of colitis by DNBS.

Discussion

The DNBS-induced experimental colitis in mice is a well-characterized transmural inflammation of the colon and may be considered a model of CD. In our previous study, we

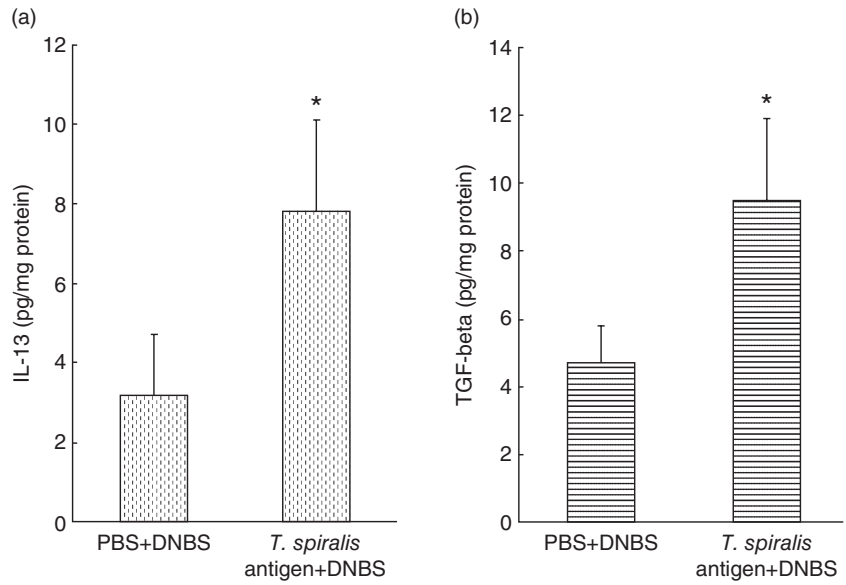


Fig. 4. Interleukin (IL)-13 and transforming growth factor (TGF)-β levels in the colonic tissues of *Trichinella spiralis* antigen-treated and phosphate-buffered saline (PBS)-treated mice following dinitrobenzenesulphonic acid (DNBS) administration. Mice were treated with *T. spiralis* antigen (100 μg in 30 μl PBS) or with PBS submucosally in the rectum 5 days before the induction of colitis by DNBS. Colitis was induced by intracolonic administration of DNBS (5 mg) and the levels of (a) IL-13 and (b) TGF-β levels in the colon tissues were evaluated by enzyme-linked immunosorbent assay on day 3 post-administration of DNBS. Each value (pg/ml) represents mean ± standard error of the mean from five mice. *Significantly higher compared with PBS-treated mice.

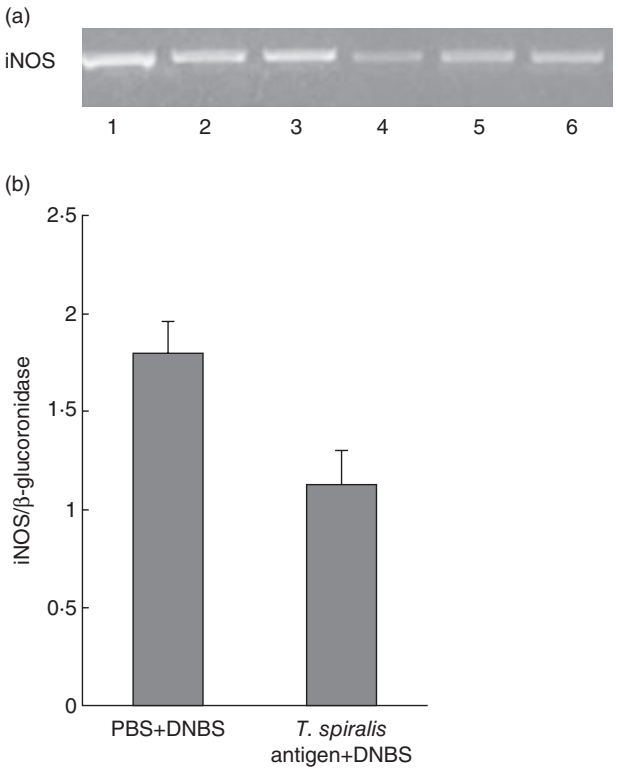


Fig. 5. (a) Inducible nitric oxide synthase (iNOS) expression investigated by reverse transcription–polymerase chain reaction (RT–PCR) within colon tissue after administration of dinitrobenzenesulphonic acid (DNBS) in *Trichinella spiralis* antigen-treated and phosphate-buffered saline (PBS)-treated mice. Mice were treated with *T. spiralis* antigen (100 μg in 30 μl PBS) or with PBS submucosally in the rectum 5 days before the induction of colitis by DNBS. Colitis was induced by intracolonic administration of DNBS (5 mg) and iNOS expression was investigated by RT–PCR on day 3 post-DNBS. β-glucoronidase was used as a positive control. (a) Lanes 1–3, PBS-treated mice after DNBS administration; lanes 4–6, *T. spiralis* antigen-treated mice after DNBS administration. (b) Ratios of iNOS band densities compared with β-glucoronidase band densities.

found that previous infection with *T. spiralis* reduced the severity of inflammation associated with DNBS-induced colitis. In the present study we have demonstrated that instead of infection with parasites, previous submucosal administration of helminth antigen ameliorated colonic inflammation significantly and reduced the mortality in this model of experimental colitis. This study has potentially exciting implications; in addition to developing a novel strategy in treating IBD this work provides us with valuable information on the immune and inflammatory mediators involved in the protection in intestinal inflammatory disorders.

There is a clear tendency to a higher incidence of IBD in developed countries compared with less-developed countries. The prevalence of IBD, particularly CD, has increased steadily in North America and western Europe in the latter part of the 20th century [7]. Studies suggest that children born to individuals who relocate from regions of low IBD frequency to areas of high disease prevalence acquire greater disease risk [9,21,22]. These observations suggest strongly an important role of environmental factors in the expression of IBD. While the incidence of CD has risen in industrialized countries, helminth infections have fallen substantially because of improved hygiene in these countries [23]. The prevalence of helminth infections in the United States has been declining for the past 60 years [7], except among new immigrants from developing countries [24]. The contrasting geographical distributions of helminth infection and CD, as well as the opposing immune response generated by helminth infections and CD, prompted consideration of a protective effect of intestinal parasitism against the development of IBD. Studies from our laboratory and others in experimental models of colitis indicate clearly that helminth infection reduces the severity of hapten-induced colonic inflammation [12,25,26]. The amelioration of colitis by helminths in these models was associated with modulation of immune response with an up-regulation in the production of IL-4, IL-13, IL-10 and TGF-β. Development of spontaneous colitis in IL-10-deficient mice is prevented or reversed by colonizing the mice with *T. muris* or *Heligmosomoides polygyrus* [7,27]. The ability of helminths to inhibit colonic inflammation suggests that helminths have therapeutic potential, and recent clinical studies provide evidence that *T. suis* ova therapy is effective in treating both CD and UC without any unfavourable side effects [13,14]. Although these clinical and experimental studies demonstrated clearly a beneficial role of helminth infection in colitis and suggest a therapeutic potential of helminths in IBD, infection with helminth eggs or worms may appear unpleasant and may not be acceptable to many. In addition to holding up the potential of helminths as immunomodulatory agents, the results of the present study provide evidence that local administration of helminth antigens can confer protection in colonic inflammation even in the absence of live infection treatment.

The beneficial effect of *T. spiralis* antigen treatment on DNBS-induced colonic inflammation was observed for all the parameters studied, including the macroscopic and microscopic indices, as well as MPO activity. MPO is an enzyme contained in the azurophilic granules of neutrophils, as well as other myeloid cells, and is used commonly as a parameter to assess inflammation [17]. Previous studies have reported extensive accumulation of neutrophils and a significant increase of MPO in DNBS-induced colitis [16,28]. IL-1β is a proinflammatory cytokine that has an important role in inflammation and is produced by many cell types of both the peripheral and central immune system, including macrophages and lymphocytes. In this study, we observed significantly lower IL-1β production in mice treated with *T. spiralis* antigen before induction of colitis by DNBS compared with the mice treated with PBS before the induction of DNBS-induced colitis.

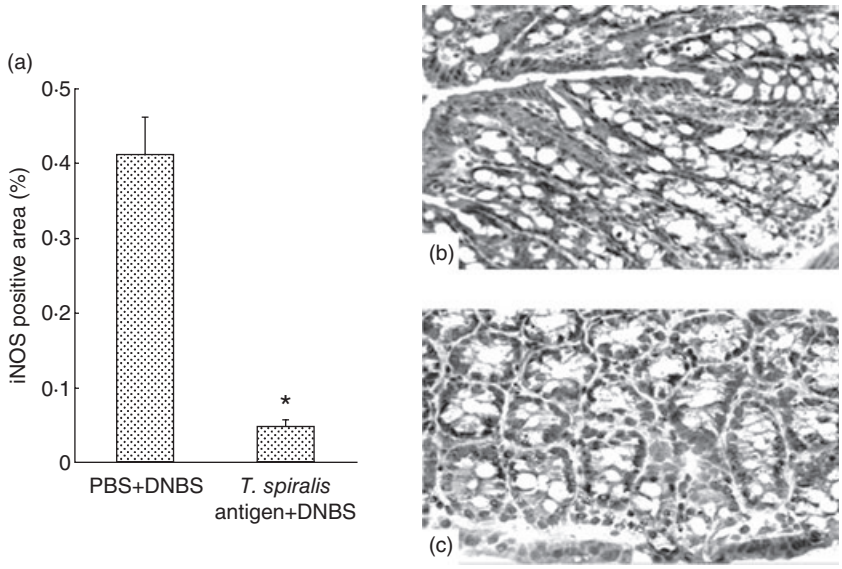


Fig. 6. Immunohistochemical localization of inducible nitric oxide synthase (iNOS) expression in colonic tissues of *Trichinella spiralis* antigen-treated and phosphate-buffered saline (PBS)-treated mice following dinitrobenzenesulphonic acid (DNBS) administration. Mice were treated with *T. spiralis* antigen (100 μg in 30 μl PBS) or with PBS submucosally in the rectum 5 days before the induction of colitis by DNBS. Colitis was induced by intracolonic administration of DNBS (5 mg) and iNOS expression was investigated by immunohistochemistry on day 3 post-DNBS. (a) iNOS-positive area. Data are expressed as the percentages of the positive staining areas of the total area. (b) iNOS immunostaining of colon tissue of PBS-treated mice in DNBS-induced colitis. (c) iNOS immunostaining of colon tissue of *T. spiralis* antigen-treated mice in DNBS-induced colitis. *iNOS-positive areas were significantly lower in *T. spiralis* antigen treated mice after DNBS compared with those in PBS-treated mice after DNBS.

The NO plays a key role as a signalling molecule as well as a regulatory molecule of the innate immune response [28]. NO is produced by almost all mammalian cells from L-arginine by one of three NOS enzymes; namely, neuronal NOS, endothelial NOS and iNOS [29,30]. iNOS is distributed widely in various cells [31] and upon exposure to stimuli such as endotoxin [lipopolysaccharide (LPS)] and proinflammatory cytokines, it is expressed rapidly and results in the production of much larger quantities of NO relative to the two other isoforms. In the gut, NO has been shown to take part in physiological and pathological events, including the pathophysiology of IBD [32–35]. Helminth infection has been shown to play an important immunomodulatory role by down-regulating iNOS expression in the gut [36]. In this study we observed reduced expression of iNOS in colonic tissues of mice which received *T. spiralis* antigens prior to induction of colitis by DNBS.

Taken together, these results indicate that rectal submucosal treatment of mice with *T. spiralis* antigen reduces the severity of colitis competently. In contrast to the reduction in MPO activity and IL-1β production we observed an up-regulation of IL-13 (Th2 cytokine) and TGF-β (regulatory cytokine) production in mice treated with *T. spiralis* antigens prior to induction of colitis by DNBS. The observation of association between increased IL-13 and reduced colonic inflammation corroborate a previous study by Elliott *et al.* [27], in which it was demonstrated that increased IL-13 production was associated with the resolution of established colitis by *H. polygyrus* in the IL-10-deficient mouse model of colitis. Recently it has been shown that after helminth exposure, LPS from commensal bacteria could regulate mucosal inflammation by stimulating TGF-β-producing T cells [37]. The observation of an up-regulation of TGF-β production in mice treated with *T. spiralis* antigens prior to induction of DNBS-induced colitis support further the notion that TGF-β is an important regulatory molecule in helminth-mediated modulation of colitis. Taken together, these findings suggest that helminth antigen treatment activated Th2 and T regulatory cells which produce IL-13 and TGF-β respectively, and subsequently reduced the occurrence of Th1-mediated inflammatory responses. In this study we have developed a novel strategy to retune the immune cell repertoire to ameliorate colonic inflammation by helminth antigens by expanding the T regulatory cell population and activating the Th2 response.

Acknowledgements

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